SPATIAL AND TEMPORAL ABUNDANCE OF GASTROPOD INTERMEDIATE HOSTS IN NORTHEASTERN MINNESOTA WITH IMPLICATIONS FOR *PARELAPHOSTRONGYLUS TENUIS* RISK IN MOOSE

A THESIS
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Advisor: Dr. Ron Moen

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Dedication

This thesis is dedicated to my amazing wife and beautiful daughter.

Abstract

Parelaphostrongylus tenuis is a nematode meningeal parasite that requires white-tailed deer primary hosts and terrestrial gastropod intermediate hosts to complete its life cycle. While white-tailed deer are the only host in which *P. tenuis* can complete its life cycle, *P. tenuis* can be lethal to moose and other cervids that accidentally ingest infected gastropods while browsing. Describing the spatial and temporal changes in gastropod abundance will increase understanding about the risk of *P. tenuis* infection by moose at the individual and population levels. We used cardboard traps and hand searches to estimate terrestrial gastropod species composition and abundance in representative vegetation communities in Northeastern Minnesota. We also tested the gastropods for *P. tenuis* infection. We also measured various environmental factors that may explain terrestrial gastropod distribution. Gastropods were most abundant in conifer and regenerating forests (11.2/m^2) while mixed forests had the lowest gastropod abundance (7.0/m^2). Gastropods were more abundant in September than June and July and none were collected in November. Seventy four gastropods were found climbing on vegetation up to three meters off the ground. 0.1% of the identified gastropods were infected with *P. tenuis.* Spatial and temporal changes in gastropod abundance could provide different risk levels of *P. tenuis* infection for moose. Our research will help to evaluate infection risk in the most common moose habitats in Northeastern Minnesota.

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Chapter 1

INTRODUCTION

The nematode *Parelaphostrongylus tenuis* is a parasite that can be fatal to moose (*Alces alces)* (Anderson 1964). *P. tenuis* infection was the probable cause of 5% of mortalities in radio-collared moose in northwestern Minnesota and >20% of incidentally-recovered moose mortalities in northern Minnesota (Murray et al. 2006, Wünschmann et al. 2014). *P. tenuis* infection causes weakness in the hindquarters, circling, tilting of the head, and increased fearlessness of humans (Anderson and Prestwood 1981). *P. tenuis* infections can also indirectly cause mortality through increased risk of predation or accidents (Lankester et al. 2007, Butler et al. 2009, Wünschmann et al. 2014). Voyageurs National Park (VNP), in northern Minnesota, maintains a stable, low-density population of about 40-50 moose (Windels 2014). *P. tenuis* infection has been documented as a mortality agent in VNP and the surrounding area (Windels 2014). Though the effect of *P. tenuis* on moose at the population level in VNP is currently unknown, previous studies suggest it is unlikely to be a major mortality source at the current low deer densities (3-6 deer/km^2) in the park (Whitlaw and Lankester 1994b).

The normal life cycle of *P. tenuis* includes white-tailed deer (*Odocoileus virginianus*) as a definitive host and terrestrial gastropods as intermediate hosts (Lankester and Anderson 1968). White-tailed deer ingest infected gastropods while foraging. Gastropods become infected with *P. tenuis* by crawling over or near infected deer feces (Lankester 2001). Only 0.1% to 4.2% of gastropods collected in surveys in

Minnesota and Ontario were infected with *P. tenuis* larvae (Lankester and Anderson 1968, Lankester and Peterson 1996). At those infection rates, a white-tailed deer would need to consume up to 1000 gastropods to ingest at least one *P. tenuis* larva (Lenarz 2009). However, Lankester and Peterson (1996) reasoned that even at such low rates of infection in gastropods, the high rates of infection seen in white-tailed deer in the region (< 91%; Slomke et al. 1995) is explained by the large volume of vegetation eaten on and near the ground over a few months in the autumn. Infection rates in white-tailed deer in VNP derived from winter fecal samples have ranged from 67-90% from the 1970s to the present (Gogan et al. 1997, VanderWaal et al. 2014). White-tailed deer are the definitive host of *P. tenuis* but moose, an aberrant host, also ingest infected gastropods during foraging and become infected. Initial signs of *P. tenuis* infection can start to appear in moose as early as 20 days after experimental infection (Lankester 2002).

Gastropods are necessary for *P. tenuis* to complete its life cycle. Therefore, knowledge of gastropod populations in VNP may help managers better understand the role of *P. tenuis* in local moose population dynamics. The distribution and the habitat preferences of terrestrial gastropods in VNP has not previously been studied. Extrapolation from studies of gastropod communities in different regions of Minnesota and the surrounding areas is possible (e.g., from northwestern Minnesota [Nekola et al. 1999] or rock outcrops in northeastern Minnesota [Nekola 2002]). However, gastropods, like many other animals, exhibit habitat preferences that result in variation in presence or density across vegetation communities or other habitat features. Few studies have

examined gastropod abundance and diversity at fine spatial scales (Moss and Hermanutz 2010).

Individual moose also demonstrate variability in habitat selection (Gillingham and Parker 2008). Large differences in cover type use can significantly alter the risk of *P. tenuis* infection to an individual moose by exposing the animal to different densities of vectors. Fine-scale habitat use derived from GPS collars can help clarify the risk of *P. tenuis* infection to individuals and populations of moose. Combined, individual differences in habitat use and variability among habitat types in gastropod diversity and abundance may result in differential risk of moose and other cervids to *P. tenuis* infection (VanderWaal et al. 2014).

In this study we surveyed terrestrial gastropod species in Voyageurs National Park's Kabetogama Peninsula. Our objectives were to 1) estimate the abundance and diversity of terrestrial gastropods found in different ecotypes, with particular focus on known vectors of *P. tenuis,* 2) document changes in gastropod abundance over the growing season, and 3) compare the use of cover types by GPS-collared moose to density of *P. tenuis* vectors to estimate the risk of encountering *P. tenuis* vectors for individual moose.

STUDY AREA

Voyageurs National Park (48.50^{o} N, 92.88^{o} W) is an 882 km^2 protected area comprised of a mixture of forested land (61%) and large lakes (39%) along the U.S.-Canada border. Moose in the park are primarily restricted to the Kabetogama Peninsula (Windels 2014), a 300 km^2 roadless area in the center of the park. Moose numbers have

remained relatively stable since the 1990s with a density in the Kabetogama Peninsula ranging from 0.14-0.19 moose/km^2 (Windels 2014). Winter white-tailed deer densities in VNP during the study likely ranged between 3-6/km^2 (Gogan et al 1997, Voyageurs National Park unpublished data). Vegetation is a mix of southern boreal and Laurentian mixed conifer-hardwood forests, comprised primarily of a mosaic of quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), white spruce (*Picea alba*), white pine (*Pinus strobus*), red pine (*P. resinosa*), jack pine (*P. banksiana*), and black spruce (*Picea mariana*)(Faber-Landgendoen et al. 2007). Soils throughout the park range from thin, sandy loams over bedrock to poorly draining clays at lower elevations (Kurmis et al. 1986). Beaver-created wetlands and associated seral stages are abundant (Johnston and Naiman 1990). Temperatures vary from -40^{o}C to 36^{o}C, with an average annual temperature of 1.4^{o}C. Mean annual precipitation is 62 cm, with most precipitation falling between May and September (Kallemeyn et al. 2003).

METHODS

We used the "ecotype"-level vegetation classification for the VNP area derived from the USGS-NPS Vegetation Map for VNP (Hop et al. 2001) and selected the 10 most common terrestrial ecotypes on the Kabetogama Peninsula to sample for gastropods. We excluded 4 ecotypes from our sampling regime because they were too wet to sample with our methods: poor conifer swamps, rich hardwood swamps, wet meadows, shrub bogs. The remaining 6 ecotypes comprised 80% of the non-aquatic vegetation communities in the Kabetogama Peninsula (Table 1). Four of the sampled

ecotypes were dry uplands (rock barrens with trees, northern spruce-fir forests, boreal hardwood forests, and northern pine forests) and two were wet lowland ecotypes (northern shrub swamp and rich conifer swamp). We randomly selected 5 polygons within each of the 6 ecotypes within a restricted area of the Kabetogama Peninsula to facilitate access to sampling sites (Figure 1). We assumed that the sites we sampled were representative of those across the entire Peninsula. We sampled for one over-night period at each site at approximately one-month intervals in each of the following periods: June 6-20, July 29-August 3, August 18-25, and September 9-14.

We used 0.25 m^2 cardboard sampling squares (50 X 50 cm) placed on ground vegetation to collect gastropods (Lankester and Peterson 1996, Hawkins et al. 1998, Nankervis et al. 2000, Maskey 2008). We randomly selected a starting sample point and direction within each polygon such that the 100-m sampling transect would fit entirely within the polygon. Ten corrugated cardboard squares were placed in a 100-m transect. Field inspection confirmed that all the sheets in each transect were in the same ecotype. The cardboard was placed directly on the soil or duff layer after rocks and branches were cleared from the sampling site. The cardboard was saturated with water and covered with a 0.36 m^2 sheet of 3-mm thick clear plastic.

Sheets were set in the morning and retrieved about 24 hours later. The wetness of each sheet was estimated as the percentage of the bottom that was visibly damp. All slugs and snails on the underside of the cardboard were collected and placed in plastic jars with damp paper towels until specimens could be identified. Identification was to the lowest taxonomic level possible using available keys (Burch 1962, Nekola 2007, and

J. Nekola pers. com.). In three cases, we lumped two closely related species together that could not be reliably differentiated by morphological characteristics: *Zonitoides nitidus* and *Z. arboreus, Nesovitrea electrina* and *N. binneyana,* and *Euconulus alderi* and *E. fulvous.* We identified potential gastropod vectors of *P. tenuis* based on a literature review (Lankester and Anderson 1968, Gleich et al. 1977, Upshall et al. 1986, Rowley et al. 1987, Platt 1989, Lankester and Peterson 1996, Whitlaw et al. 1996, Nankervis et al. 2000, Lankester 2001).

We considered the 100-m sample transect the sample unit and tested for the effects of ecotype and sample period on abundance of gastropod groups (total gastropods, snails only, slugs only) using factorial ANOVA. We also tested for an interaction between ecotype and sampling period. We used Bonferroni corrections when making post-hoc comparisons between main effects (ecotype and sample period). We considered results significant at the P=0.05 level.

We obtained GPS locations at 15-minute intervals from 11 adult moose (9F:2M) wearing GPS collars during the period June-September 2010. Spatial data was displayed using ArcGIS 10.1 with ArcGIS Spatial Analyst (ESRI, Redlands, CA, USA 2012), while home ranges were calculated in the Geospatial Modeling Environment (2012 Spatial Ecology LLC) running via ArcGIS 10.1 and R 3.0.1. We calculated the proportion of moose locations that were located in each ecotype for individual moose. We calculated a relative measure of *P. tenuis* transmission risk to moose in different VNP ecotypes by comparing the abundance of gastropods in each ecotype to the habitat use of moose in each ecotype. Mean monthly moose habitat use (i.e., proportion of all locations within

an ecotype) did not vary much between June to September; all differences were < 5% between months for any ecotype. We therefore used the mean proportion of use for the entire June-September period to estimate an overall risk of *P. tenuis* infection by ecotype during summer.

We also evaluated variation in relative risk of *P. tenuis* infection to individual moose. Risk Value was calculated by multiplying the proportion of each ecotype used by a moose by the mean density of potential *P. tenuis* gastropod vectors sampled in each ecotype. We scaled the Risk Value for each moose to the highest individual Risk Value to compare relative risk of infection among individual moose. Our indices of risk of *P. tenuis* infection assume that 1) gastropod infection rates (i.e., proportion of gastropods infected with *P. tenuis* larvae) did not vary among gastropod species, among habitat types, or over the sampling time, and 2) the likelihood of a moose ingesting a potentially infected vector gastropod in a given ecotype is proportional to the density of known vectors of *P. tenuis* in that ecotype. Our index of risk does not consider morbidity or mortality for infected moose, because the severity and duration of the infection can be highly variable (Lankester 2002, 2010).

RESULTS

We collected 6,595 gastropods representing 9 families and 25 species (3 slug species and 22 snail species; Table 2). We completed classification for 62% of slugs and 50% of snails collected. Because of time constraints, gastropods from three of the five replicates for each ecotype were identified. We could not identify 585 (9%) of the gastropods collected because they were damaged beyond recognition during collection

and storage or they were juveniles, which can be difficult to accurately identify even to the family level (J. Nekola, pers. comm.). The total number of snails sampled per m^2 (including unidentified) increased from July to September for all ecotypes combined (ANOVA, $F_{3,29} = 8.7$, $P < 0.001$). The treed rock barren cover type had the lowest snail density ($7.1/m^2$) for all sampling periods combined. Northern pine forest and northern spruce-fir forest ecotypes had the most snails for all periods combined and increased from 7.3 and 10.2 snails per m^2, respectively, in July to about 23.7 and 22.8 snails per m^2 in September (Figure 2).

Slug densities (including unidentified) were more variable over the growing season than were snail densities (Figure 3). Overall, slug density was relatively constant over time within each ecotype, and at lower densities than snails. Slug densities, in all four sampling periods combined, were lowest ($1.3/m^2$) in the rich conifer swamp ecotype and highest in the northern pine forest ($6.2/m^2$) and northern spruce-fir forest ($6.9/m^2$). Northern shrub swamp ($3.3/m^2$) and rich conifer swamp ($1.3/m^2$) had significantly lower slug densities than the other 4 ecotypes (ANOVA, $F_{5,29} = 20.88$, $P < 0.001$).

Cardboard wetness increased from June to September (ANOVA, $F_{3, 29} = 165.8$, $P < 0.001$). For example, mean wetness during Survey 1 was 47% but was 90% in Survey 4. Within ecotypes, cardboard wetness in the treed rock barren ecotype was significantly lower (51%) than the other 5 ecotypes (range 75-82%; ANOVA, $F_{5, 29} = 44.3$, $P < 0.001$).

Eight of the collected species are known vectors of *P. tenuis* and comprised 32% of the gastropods caught. The slug *Deroceras laeve* was the most common vector

collected, comprising 26% of total captures. *D. laeve* was present in every ecotype but was most common in the northern spruce-fir forests ecotype. Two other slug vectors found were *Pallifera hemphili* and *Deroceras* sp., a congener of *D. laeve* we could not identify to species but assumed was also a *P. tenuis* vector. The snails *Discus cronkhitei, Zonitoides nitidus+arboreas, Strobilops* spp., and *Cochlicopa* sp., also known vectors of *P. tenuis,* represented about 11% of gastropods collected and were found across all sample sites and surveys (Table 2).

Risk of *P. tenuis* infection was highest in northern spruce-fir forests (Figure 4). The northern spruce-fir ecotype had the highest mean use by moose (35% of total locations) and also had the second highest estimated density of *P. tenuis* vectors. Treed rock barrens had the fourth highest use by moose (8%) but also had the third highest *P. tenuis* vector density, thus treed rock barrens are a moderate risk ecotype. Boreal hardwood forests were also a moderate risk ecotype due to a combination of relatively high moose use and low vector density. Rich conifer swamps and northern shrub swamps were low risk ecotypes because of their relatively low use by moose (5% and 7%) and low densities of *P. tenuis* vectors (Figure 4).

Not surprisingly, moose also displayed variability in their individual risk of infection as a result of individual differences in habitat use (Table 3). Ten of 11 moose had Relative Risk scores between 0.68 and 1.0, i.e., Relative Risk differed by ≤32% for most moose in our sample. Moose V09, however, spent relatively little time in

gastropod rich habitats and therefore it had a much lower risk of *P. tenuis* infection (0.21) relative to the other moose we sampled.

DISCUSSION

Gastropod density, and more specifically density of known vectors of *P. tenuis*, differed among the ecotypes we sampled and between the sampled periods. Similar to previous studies, ecotypes of mixed conifer-deciduous forest types had the highest gastropod densities (Gleich et al. 1977, Kearney and Gilbert 1978, Nankervis et al. 2000). Our finding of increasing density of gastropods and potential *P. tenuis* vectors from summer to fall is also consistent with a previous studie in northern Minnesota (Lankester and Peterson 1996). *D. laeve* was the most abundant gastropod found in our study, and therefore likely the most important vector of *P. tenuis* in our area. Most larvae in infected gastropods are also likely to be in the infective stage (i.e., third stage) by early July (Lankester and Peterson 1996). Because our gastropod sampling took place between mid-June and September, infected gastropods sampled in our study would likely be carrying the infective 3rd stage larvae.

The cardboard sampler method we used is meant to provide a relative measure of gastropod diversity and abundance, and therefore it is critical that cardboard samplers be as uniform as possible in terms of shape, thickness, wetness, etc. All cardboard sheets used in our study were saturated with water at the time of deployment. The cardboard dried out at different rates depending on habitat features (e.g., soil moisture, rockiness, etc.) and weather (e.g., dry and windy vs. calm and humid). Cardboard wetness varied from 0 to 100% when cardboard samplers were

checked for gastropods. This wide variation in wetness could skew the estimates of gastropod abundance because gastropods are less likely to be found on dry cardboard (Boag 1982). Variation in cardboard wetness could be minimized by setting the cardboard after the warmest part of the day and checking them before the warmest part of the next day. This could be especially important in the longer and warmer days in July and early August. Since the cardboard sheets do not dry instantaneously, gastropods that are attracted to the wet cardboard may concentrate themselves on the wet areas as the cardboard dries. Past studies have found lower gastropod abundances in the early summer (Lankester and Anderson 1968, Kearney and Gilbert 1978, Lankester and Peterson 1996). These studies did not report on the wetness of cardboard sheets, however, and may be biased low if sheets were drier in the early summer. Cardboard samplers may underestimate the total density of gastropods in an area, as the numbers of gastropods in the soil underneath cardboard samplers have been reported to be significantly higher than those attached to cardboard itself (Hawkins et al. 1998).

By combining information about gastropod density and relative moose use of different habitats, we assessed the relative risk of *P. tenuis* infection for moose that may use individual habitat types (Figure 4). We likewise calculated Risk Values for individual moose (Table 3). These methods can also be used to compare risk of infection between different geographic areas or populations. However, we caution that the assumptions associated with our methods need to be considered carefully. For example, seasonal variation in infection rates in gastropods hosts has not been well studied (Lankester and

Anderson 1968, Kearney and Gilbert 1978, Lankester and Peterson 1996). Higher deer densities have been correlated with increased infection rates of gastropods (Lankester and Peterson 1968) and moose (Whitlaw and Lankester 1994a) at larger spatial scales. A recent study did not find a correlation between deer abundance and *P. tenuis* infection in white-tailed deer at smaller spatial scales within VNP (VanderWaal et al 2014), although the range of deer abundances across sites was limited.

Risk of *P. tenuis* infection will vary among individual moose because of differences in habitat use within their respective home ranges. Risk of infection will also be influenced by landscape composition and the availability of different habitats within an area. For example, the western half of the Kabetogama Peninsula has more area covered by the higher risk boreal hardwood and northern spruce-fir ecotypes. By contrast, the eastern half of the park contains more of the drier, low risk treed rock barrens and northern pine ecotypes. Moose in the western portion of the park therefore may be at higher risk of infection than in the eastern portions. VanderWaal et al. (2014) found that *P. tenuis* infection rates in white-tailed deer increased as the proportion of vector-rich habitats such as mixed conifer-hardwood forest increased within the local area.

While our methods only considered coarse habitat use in our Risk Index, moose behavior within individual ecotypes is likely also important. Moose may prefer to bed in certain ecotypes (e.g., in lowland habitats in hot weather) and feed in others (Peek 1997). Even if gastropods are abundant in certain ecotypes, the risk of *P. tenuis* infection should be less in areas less preferred for foraging. Risk of *P. tenuis* infection in moose

also may be affected by factors such as individual preferences for forage choice, previous exposure to *P. tenuis*, health status, genetics, body mass/longevity (Ezenwa et al. 2006), and other factors we have not considered here.

Table 1. Area (km^2) and % total area[1] covered by each of six terrestrial vegetation ecotypes sampled on the Kabetogama Peninsula, Voyageurs National Park (VNP), June-September, 2011.

Ecotype	Area (Km^2)	%
Northern Spruce-Fir Forest	66	23
Boreal Hardwood Forest	62	21
Northern Pine Forest	52	18
Treed Rock Barrens	39	13
Northern Shrub Swamp	8	3
Rich Conifer Swamp	5	2
Total	232	80

[1]Area calculations exclude lakes, ponds, etc. Ecotype classifications are according to the US-National Vegetation Classification System applied to VNP (Hop et al. 2001).

Table 2. Composition of terrestrial gastropods[1] collected in Voyageurs National Park, June-September, 2011.

Group	Family	Species	Count	% Total Captures
P. tenuis Vectors				
Slug	Limacidae	*Deroceras laeve*	906	26.0
Slug	Limacidae	*Deroceras sp.* (but not *D. leave*)	13	0.4
Slug	Philomycidae	*Pallifera hemphili*	6	0.2
Snail	Endodontidae	*Discus cronkhitei*	55	2.0
Snail	Strobilopsidae	*Strobilops* spp.	145	4.0
Snail	Valloniidae	*Cochlicopa* spp.	6	0.2
Snail	Zonitidae	*Zonitoides (nitidus+arboreas)*	159	4.6
Total			1290	37.4
Non-vectors				
Snail	Endodontidae	*Helicodiscus parallelus*	7	0.2
Snail	Endodontidae	*Punctum californicum*	7	0.2
Snail	Endodontidae	*Punctum minutissimum*	2	<0.1
Snail	Endodontidae	*Punctum* spp.	4	0.1
Snail	Oxychilidae	*Nesovitrea (electrina+binneyana)*	105	3.0
Snail	Pupillidae	*Columella simplex*	6	0.2
Snail	Pupillidae	*Gastrocopta pentodon*	6	0.2
Snail	Pupillidae	*Gastrocopta sp.*	11	0.3
Snail	Pupillidae	*Vertigo* spp.	319	9.0
Snail	Pupillidae	*Unknown*	143	4.0
Snail	Succineidae	*Oxyloma retusa*	19	0.5
Snail	Valloniidae	*Cochlicopa lubricella*	11	0.3
Snail	Valloniidae	*Zoogenetes harpa*	62	2.0
Snail	Zonitidae	*Euconulus (alderi + fulvous)*	638	18.0
Snail	Zonitidae	*Guppya sterkii*	6	0.2
Snail	Zonitidae	*Striatura milium*	29	0.8
Snail	Zonitidae	*Striatura exigua*	7	0.2
Snail	Zonitidae	*Striatura ferrea*	6	0.2
Snail	Zonitidae	*Vitrina limpida*	326	9.0
Snail	Zonitidae	Unknown	461	13.0
Total			2175	61.4

[1]Gastropod species were identified to the lowest taxonomic level possible. We classified 62% of slugs and 50% of snails collected.

Table 3. Proportional habitat use and individual risk[1] of moose encountering *P. tenuis* infected gastropods in the Kabetogama Peninsula, Voyageurs National Park, June-September 2010.

	Proportion Habitat Use							
Moose #	Northern Pine Forest	Northern Spruce-Fir Forest	Treed Rock Barren	Boreal Hardwood Forest	Northern Shrub Swamp	Rich conifer Swamp	Risk Value	Relative Index of Risk
V05	0.43	0.18	0.02	0.24	0.00	0.03	9.50	1.00
V06	0.42	0.10	0.02	0.17	0.03	0.05	8.96	0.94
V07	0.09	0.34	0.11	0.18	0.07	0.04	8.91	0.94
V14	0.05	0.35	0.14	0.19	0.10	0.04	8.76	0.92
V07	0.04	0.37	0.10	0.18	0.06	0.03	7.80	0.82
V10	0.05	0.33	0.10	0.19	0.06	0.03	7.66	0.81
V18	0.07	0.19	0.26	0.19	0.02	0.00	7.64	0.80
V17	0.13	0.25	0.08	0.18	0.03	0.02	7.44	0.78
V12	0.10	0.32	0.03	0.21	0.06	0.05	7.23	0.76
V08	0.01	0.37	0.00	0.27	0.02	0.01	6.49	0.68
V09	0.00	0.17	0.00	0.13	0.09	0.11	2.04	0.21

[1]Risk value is calculated by multiplying the proportion of each ecotype used by a moose by mean density of *P. tenuis* gastropod vectors sampled in each ecotype. The Relative Index of Risk is the Risk Value scaled to the highest Risk Value found for an individual moose in 2010 (i.e., Moose V05).

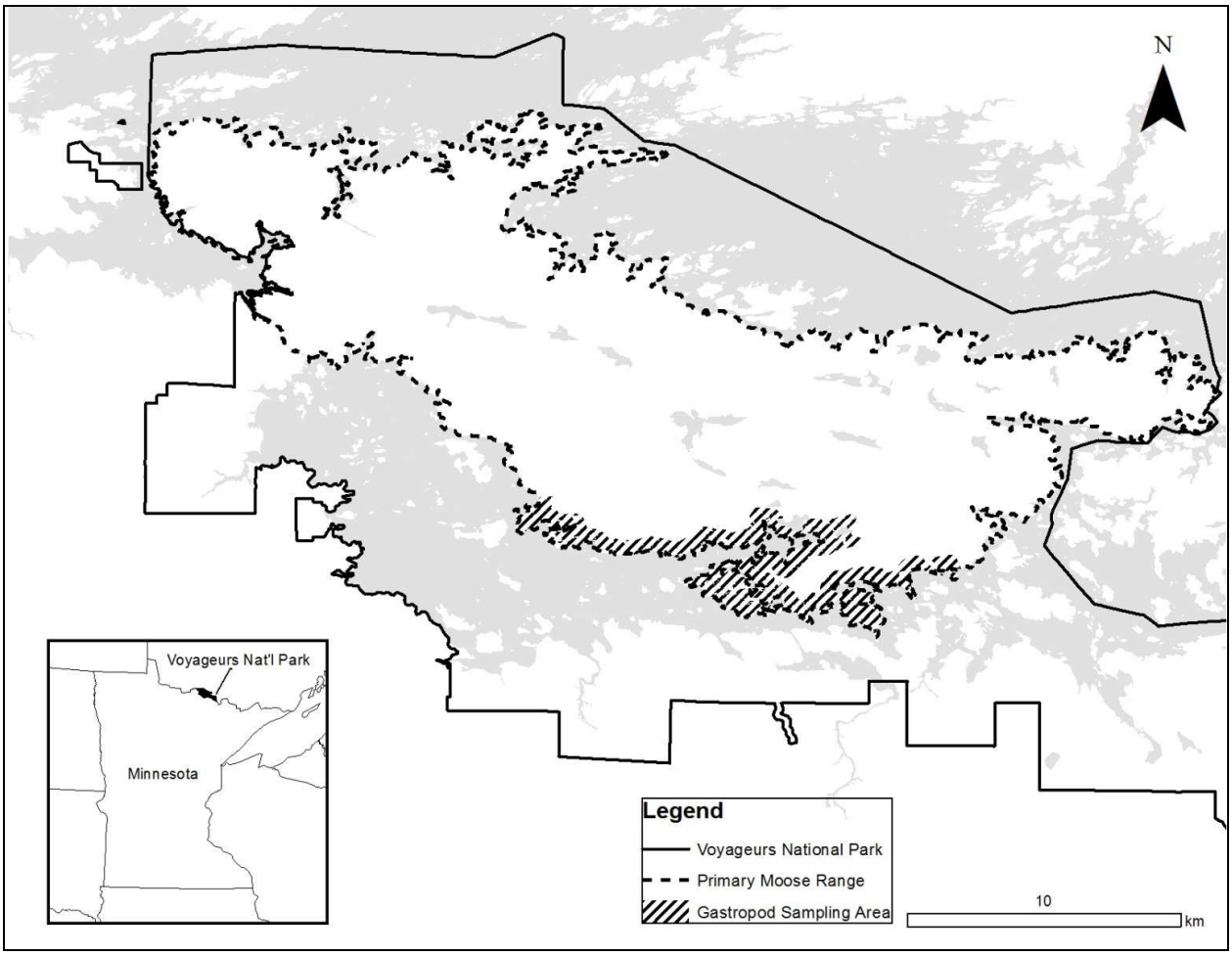

Figure 1. Primary moose range (dashed line) and terrestrial gastropod sampling area (hatched area) in Voyageurs National Park, Minnesota, from July 6-September 14, 2010.

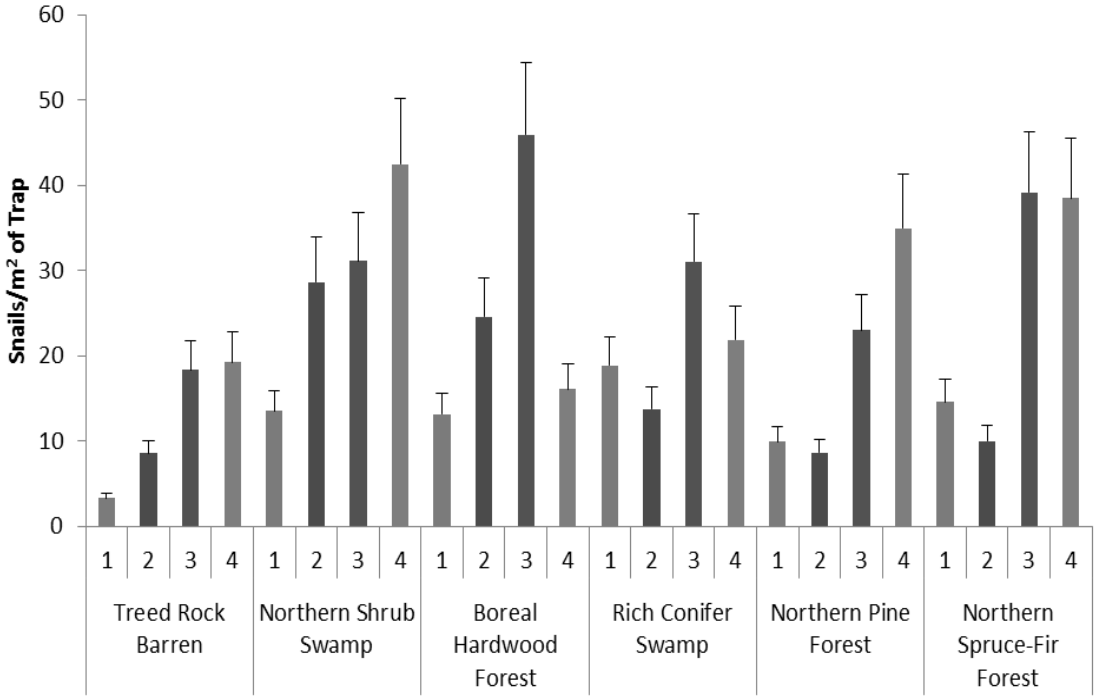

Figure 2. Mean (+SE) number of snails/m^2 (including unidentified) sampled in each of 6 ecotypes for one over-night period in each of four sampling periods[1] in June-September, 2011 in Voyageurs National Park.

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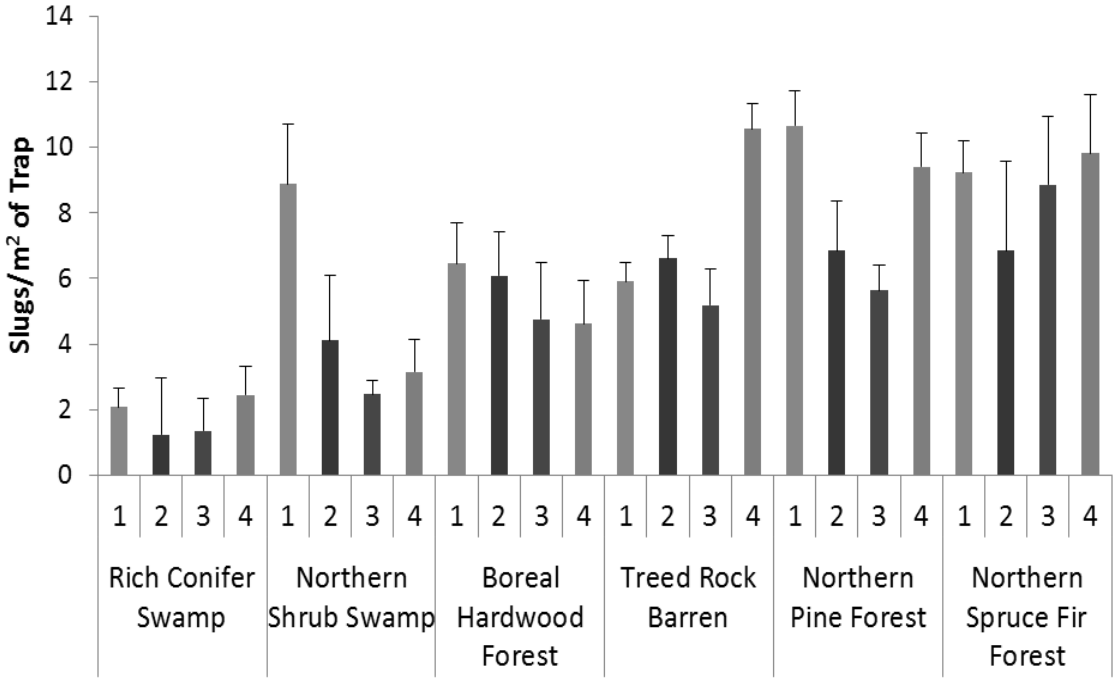

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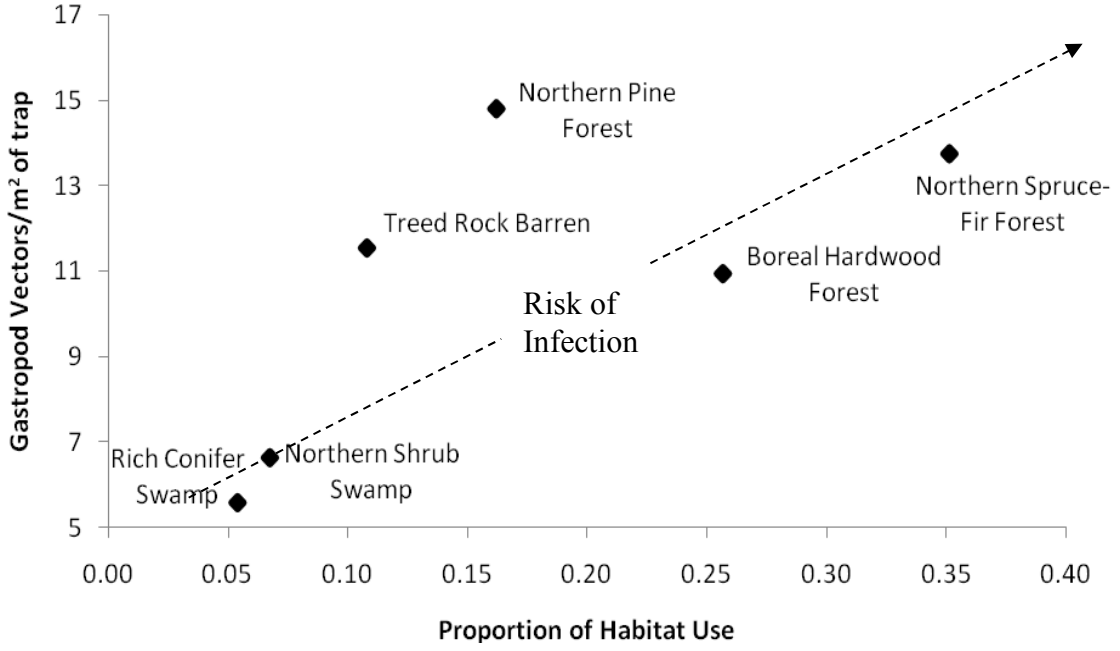

Figure 4. Relative risk of moose encountering *P. tenuis* gastropod vectors in six ecotypes in the period July to September, 2010, in Voyageurs National Park.

Chapter 2

Introduction

Parelaphostrongylus tenuis is a nematode meningeal parasite that requires white-tailed deer (*Odocoileus virginianus*) as a primary host and gastropod intermediate hosts to complete its life cycle. While white-tailed deer are the only host in which *P. tenuis* can complete its life cycle, moose and other cervids will also accidentally ingest infected gastropods while browsing (Lankester 2007, Lankester 2010, Suominen 1999). Infected white-tailed deer drop first stage *P. tenuis* larva in feces. This larva is transmitted to terrestrial gastropods, either slugs or snails, as they crawl over the feces while searching for food (Lankester 1997). Inside the gastropod, *P. tenuis* grows into infective third stage larvae which are incidentally ingested by white-tailed deer while browsing.

The transmission of *P. tenuis* between gastropods, white-tailed deer, and moose (*Alces alces*) varies depending on factors such as winter temperatures and lengths, presence of suitable hosts, and local habitat. Winters with highly variable temperatures reduce *P. tenuis* larvae survivorship from 76% to 16% (Forrester 1998). *P. tenuis* is found from eastern North America westward to the central plains in the Dakotas and eastern Saskatchewan. Dry, arid conditions in the central plains act as an environmental barrier to gastropods and also have lower deer densities (Lankester 2010, Maskey

2008). Gastropods are found in lower densities in open areas with low canopy cover (Platt 1989, Upshall 1986).

Variations in climate limit gastropod activity level which changes the prevalence of *P. tenuis* transmission. Longer or colder winters decrease the opportunities for gastropods to be infected by *P. tenuis* by reducing the length of their active season. Gastropods can survive over winter, but they are not active during freezing temperatures. Moist climates increase the prevalence of *P. tenuis* in gastropods because the larvae are able to survive longer in the deer feces and surrounding soil with increased infectivity, increasing the likelihood of gastropod encounters (Lankester 1968, Shostak and Samuel 1984). White-tailed deer also have higher infection rates in winters with <20 cm of snow in winter yards (Peterson et al. 1996). Gastropods have increased reproductive success and a higher survival rate in wet climates or particularly wet years (Hawkins 1997). Wet deer feces, both with and without *P. tenuis* larva present, attracted at least one species of host snail (Garvin 2005).

P. tenuis causes “moose sickness”, which is one of the less understood causes of moose fatality. Although the direct negative effects of the parasite on individual moose are understood, the effects on moose population dynamics are not known. The infected animal is weak, fearless, lethargic, semi-blind, and unable to stand (Anderson and Prestwood 1981). These symptoms can either be directly or indirectly fatal. Sick moose are more likely to be hit by cars, taken as prey, or fail to ingest enough food (Lankester

1997). Symptoms can appear 20 days after infection (Anderson and Prestwood 1981). Female moose survive the presence of *P. tenuis* longer than males, with 76% of infected moose over the age of three being female (Lankester 2010).

Although white-tailed deer are necessary for *P. tenuis* to persist, an increase in deer density does not necessarily cause extirpation of moose from an area (Anderson and Prestwood 1981, Schmitz 1994, Lenarz 2009). Deer densities of >5/km^2 have been associated with negative effects on moose populations (Whitlaw and Lankester 1993). The deer populations in northeastern Minnesota are estimated at 8-18/km^2 (Lenarz and Grund 2011). Variations in gastropod density are a strong factor in the effects of increasing deer density on local moose populations (Schmitz 1994).

In this study we surveyed terrestrial gastropods in the Superior National Forest in Lake County, MN. Our objectives were to 1) find the gastropod community composition and density in four major terrestrial moose habitats, 2) test for changes in gastropod community structure between years and seasons, 3) measure the effects of microhabitat on gastropod communities, and 4) find the prevalence of *P. tenuis* in different gastropod species and habitats.

Study Area

The area used for sampling is based on the location of several radiocollared moose in the Superior National Forest near Finland and Isabella, MN (47.6172° N, 91.3550° W). This area is part of the Laurentian mixed forest province characterized by

black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), jack pine (*Pinus banksiana*), red pine (*P. resinosa*), and white pine (*P. strobus*). Paper birch (*Betula papyrifera*), quaking aspen (*Populus tremuloides*), red maple (*Acer rubrum*), and sugar maple (*A. saccharum*) are also common throughout the region (Tester 1997). Average precipitation is 28 to 32 inches with 12 inches coming from June to August as rain. The average winter temperature is 8°F and the average summer temperature is 62°F (MNDNR 2008). White-tailed deer densities range from 8 to 18 deer/km^2 within the study area (Lenarz and Grund 2011).

Methods

Gastropod Sampling: We sampled the four most common terrestrial forested habitat types used by moose in northeastern Minnesota: coniferous forests, deciduous forests, mixed forests, and regenerating forests (Lenarz and Grund 2011). Possible habitat stands were located using ArcMap 10 with Land Use Land Cover raster data (MNDNR 2007 [cited April 2013]). We also defined each forest type in the field by its tree composition. Deciduous forests had >75% deciduous trees, conifer forests had >75% coniferous trees, mixed forests had <75% of each tree group, and regenerating forests were disturbed within the last 20 years based primarily on tree size and recent satellite maps (USGS 1992 [cited December 2014]).

Eight stands for each habitat were selected and four were randomly chosen for sampling (Figure 5). We had 4 transects in each habitat in 2013 and 2014, except in

2013 we had three conifer transects. In 2014 we randomly selected two transects from each 2013 habitat to resample and designated two new transects for each habitat. We sampled 8 transects per week during the sampling months. Transects consisted of ten cardboard sheets (50 X 50 cm) set 10 m apart (Lankester and Peterson 1996, Hawkins et al. 1998, Nankervis et al. 2000, Maskey 2008). Each week the sheets were moved 1 m to prevent over sampling of the same area of soil. The entire transect was contained in a single stand of each habitat type.

In eight transects, two of each cover type, we set two additional cardboard sizes (35 X 35 cm and 20 X 20 cm). The smaller cardboard sheets were placed two meters apart from the standard cardboard sheets. We collected all gastropods that were attached to the cardboard sheets in order to compare gastropod density estimates using different sizes of cardboard. However, they were not included in gastropod density or infection analysis between cover types and seasons.

The cardboard sheets were saturated in water the afternoon before we sampled for gastropods. We placed the wet sheets directly on the litter or duff layer, removing any large sticks or rocks. The sheets were left overnight and checked for gastropods between 6 A.M. and 12 P.M. the following day. We collected all gastropods attached to the bottom or top of the cardboard sheet. All gastropods were placed in empty sampling jars and taken back to the lab for processing. Along with gastropods, we recorded the percent wetness of each cardboard sheet based on visual estimation to

the nearest 10 percent and tested the soil moisture levels under each trap using a Ground Work dual probe soil meter.

Slugs were identified the day after collection because they can deteriorate rapidly. Snails were placed in collection vials in 70% ethanol to be identified later. Gastropods were identified to the lowest taxonomic level possible using Birch (1962), Barthel (2000), and Nekola (2007). For two genera we grouped together closely related snail species: *Zonitoides nitidus* and *Z. arboreus,* and *Euconulus alderi* and *E. fulvus*. Specimens from June and July 2013 were unable to be identified do to a storage error and were not included in ID analysis. However, we were able to use abundance values from those months.

***P. tenuis* larvae testing:** *P. tenuis* larvae counts in gastropod species were performed using an artificial digestion procedure (Lankester and Peterson 1996, Nankervis et. al 2000). Gastropods were batch digested by species and transect. Each batch was crushed with a wooden dowel and suspended on wire mesh in a plastic beaker of artificial digestion fluid (0.7 ml of HCl and 0.6 g of pepsin powder per 100 ml of DI water). After 24 hours at room temperature (21°C), the batch was separated into two petri dishes, one containing the digestion fluid and the other containing the remains of the gastropods in 3 ml of fluid.

The contents of the petri dishes were examined under a dissecting microscope (16X) for the presence of *P. tenuis* larvae. Larvae were identified using Anderson (1963).

Habitat Classification: Eight environmental measurements were taken at each transect in order to find correlations between microhabitats and gastropods. We used the average of measurements at traps 1, 5, and 10 in each transect for calculations. Canopy cover was measured using a densiometer to average four readings at the cardinal directions at each location. Ground cover was estimated by the percent (to the nearest 10%) of ground vegetation in square 1 m^2 plots. Tree density was calculated by counting the number of trees in 10 X10 m plots and converting to trees/hectare. Basal area was measured with a wedge prism (BAF 20) (Hale 2006 [cited July 2013]). Duff and leaf litter thickness was measured to the nearest millimeter. Soil type was measured using Brewer's (1982) simplified key to mineral soil texture. Soil pH was found using Luster Leaf Rapitest pH testers (Luster Leaf, 2014). These measurements were taken in early August before leaves began to fall.

Statistics: Each transect was considered a sampling unit. We tested for the effects of habitat type, year, and habit X year on gastropod abundance (snails, slugs, and total gastropods) using factorial ANOVA. We used Bonferroni corrections when making post-hoc comparisons between main effects (habitat and time). We considered results significant at the P=0.05 level. We used best subset regression analysis to test the effects of each environmental habitat measurement on gastropod abundance and develop a predictive model for gastropod abundance. AICc, Cp, and Adjusted R^2 were

used to select the best fitting model. A log transformation was used for all variables after starting analysis with no transformations.

Results

We collected 5,618 terrestrial gastropods, 3,701 in 2013 and 1,917 in 2014 (Figure 6). We collected 3,471 (62%) slugs and 2,147 (38%) snails. These represented 17 gastropod species, 4 slug species and 13 snail species (Table 4). The most common gastropod collected was the slug *Arion subfuscus* making up 44% of the identified specimens (Table 4). *A. subfuscus* was the most common gastropod in every habitat, but tended to be most abundant in deciduous forests (ANOVA, $F_{3,161} = 2.57$, $P = 0.0565$). The slug *Deroceras laeve* was the second most common species (12%) and was found equally in all habitats. *Discus cronkhitei* was the most common snail identified and tended to be more common in regenerating forests. The only snail found more frequently in conifer forests than other habitats was *Euconulus* spp. (ANOVA, $F_{3,161} = 3.57$, $P = 0.0155$). *Zoogenetes harpa* was most abundant in regenerating forest (2.6/m^2) (ANOVA, $F_{3,161} = 2.96$, $P = 0.034$).

D. laeve stayed at a relatively low density (3.4 – 4.9/m^2) until October (10.3/m^2) (ANOVA, $F_{5,161}$, = 4.22, $P = 0.0012$) (Figure 9). *Z. harpa* also peaked in September and October (7.3/m^2) compared to May and June (2.8/m^2) (Figure 5) (ANOVA, $F_{5,161}$, = 3.48, $P = 0.0051$). The only species that varied between 2013 (2.0/m^2) and 2014 (0.6/m^2) was *Vitrina angelicae* (ANOVA, $F_{3,161} = 20.19$, $P = 0.001$). *A. subfuscus* tended to have higher

densities in May (18.5/m^2) and October (15.8/m^2), with a decrease in the summer months of July (10.7/m^2) and August (11.7/m^2).

Conifer forests (10.0/m^2), regenerating forests (11.0/m^2), and deciduous forests (10.8/m^2) had similar gastropod densities. Slugs had the highest densities in deciduous forests (7.5/m^2) (Figure 6). Snails were most dense in regenerating forests (5.8/m^2) (Figure 7). Mixed forests tended to have the lowest densities for both slugs (4.8/m^2) and snails (2.9/m^2). However, there was no significant difference amoung habitat types for gastropod densities (ANOVA, $F_{3,30} = 1.09$, $P = 0.369$), snail densities (ANOVA, $F_{3,30} = 2.05$, $P = 0.13$), or slug densities (ANOVA, $F_{3,30} = 0.74$, $P = 0.539$).

There were also no significant differences in gastropod abundance between 2013 and 2014 (Two sample T-test, $t_{29} = -0.43$, $P = 0.667$). Conifer forests had more slugs in 2013 (6.3/m^2) than in 2014 (5.0/m^2) (Figure 6). Deciduous forests had lower gastropod densities in 2013 (6.2/m^2) than 2014 (8.9/m^2). Snails did not vary more than 1/m^2 among all habitats between 2013 and 2014 (Figure 7). September had the highest densities of both slugs (10.5/m^2) and snails (5.1/m^2) with combined data for 2013 and 2014. June (9.8/m^2) had a small peak of gastropod densities before declining in July (8.8/m^2). No gastropods were found in November (Figure 8).

Four gastropods were infected with *P. tenuis* larvae (0.1%) (Table 4). Two gastropod species had larvae, 1 *D. laeve* (0.16%) and 3 *D. cronkhitei* (0.2%). The *D. laeve* was found in the deciduous forest cover type. One infected *D. cronkhitei* was in the

deciduous forest cover type while the other two infected *D. cronkhitei* were found in the regenerating forest cover type. No infected gastropods were found in conifer forest or mixed forest cover types.

Gastropod density estimates increased as the size of cardboard sheet decreased (ANOVA, $F_{2,573} = 11.91$, $P = <0.001$). The estimate using 50 X 50 cm sheets was 8.6 gastropods/m^2 while the estimate using 20 X 20 cm sheets was 18.9 gastropods/m^2 (Figure 10). The 35 X 35 cm sheets (11.0 gastropods/m^2) did not vary from the 50 X 50 cm sheets, but both larger sizes were significantly smaller density estimates than the 20 X 20 cm sheets.

Four variables were included in a multiple linear regression model for gastropod abundance: basal area, tree density, ground cover, and canopy cover. The resulting model ($R^2 = 0.2245$, Cp = 3.9, AICc = 0.16) was:

$$\text{Gastropod/m}^2 = 4.6 - 6.2[\log(\text{BTA})] - 5.4[\log(\text{Canopy Cover})] + 6.9[\log(\text{Tree Density})] + 5.4[\log(\text{Ground Cover})].$$

Discussion

Gastropods were found ubiquitously in all habitat types. All but the five rarest species (<7 specimens) were found in all four habitats. Snails and slugs are very adaptable to different habitats and climates and tend to be found throughout a landscape, even in certain deserts (Schmidt-Nielsen et al. 1971, Nekola et al. 2002). The most ubiquitous and common species we found was the non-native slug *Arion*

subfuscus. *A. subfuscus* is a very aggressive slug that is much larger than most native slugs in northern Minnesota, growing to 70 mm in length (Burch 1962). They get particularly aggressive towards other gastropods during the summer when shelter and food are more limiting (Rollo and Wellington 1979). *A. subfuscus* are able to reproduce in large numbers (50 eggs/clutch) compared to the native *Deroceras laeve*(<30 eggs/clutch) (Quick 1960). *A. subfuscus* has damaged large areas of boreal forests by feeding heavily on tree seedlings and other small plants (Nystrand 2000, Fritz 2001, Cameron 2009).

Because of the aggressive nature of *A. subfuscus*, they are able to reduce the populations of other gastropod species. Twenty five species of gastropods were identified in Voyageurs National Park with no *A. subfuscus* present (Cyr et al. 2014). However, in the Superior National Forest, only 17 species were identified, with *A. subfuscus* comprising 44% of the individuals. There could be other reasons for lower gastropod species diversity being found in Superior National Forest, including more human use and development. However, based on the high number of *A. subfuscus* found in SNF and their ability to negatively affect native gastropod species, they are likely contributing to a decrease in native species.

Since gastropod density estimates had an inverse relationship with cardboard size, it appears that the wet cardboard may act as an attractive force for gastropods (Figure 10). Gastropods likely move from surrounding areas to the protective and moist

climate provided by the wet cardboard. This is probably increased in hot, dry conditions. For this reason, it is important to use a standard cardboard size within studies and comparing between studies. Although it is more efficient to use smaller cardboard sheets, density estimates are more consistent with previous studies using larger cardboard. A follow up study could use 1 m^2 cardboard sheets to find gastropod density without having to scale to 1 m^2.

Our *P. tenuis* infection rates (0.1%) in gastropods was similar to Lankester and Peterson (1996) infection rate in northeastern Minnesota (0.08%) even though our sample size was lower (3,794 compared to 12,096). Although *A. subfuscus* has been found infected in low percentages (Whitlaw et al. 1996), they have very large bodies and thick tissue which makes it difficult to detect the presence of *P. tenuis* larvae (Nankervis et al. 2000). Since *A. subfuscus* was the most abundant species, this could have lowered our average infection rates. Infection rates would be slightly higher (0.18%) excluding *A. subfuscus* from analysis.

All four infected gastropods were collected in August and September, when gastropod densities were highest. *P. tenuis* larvae mature to third stage (infective) larvae in July (Lankester and Peterson 1996). With increased gastropod densities and infection rates in September, white-tailed deer and moose could become infected at higher rates during this period.

Both infected species we found, *D. cronkhitei* and *D. laeve*, are common hosts of *P. tenuis* (Platt 1989, Lankester and Peterson 1996, Whitlaw et al. 1996, Nankervis et al. 2000). Both deciduous forests (0.16% infection) and regenerating forests (0.21% infection) had two infected gastropods. Because moose and deer heavily browse deciduous and regenerating forest habitats, they may be important sites for infection. Other species that we collected are known hosts in other areas, and were not captured in high enough numbers to detect infections. These known host species include *Euconulus* spp*., Zonitoides arboreus, Cochlicopa lubric, Succinea ovalis, Strobilops affinis,* and *Anguispira alternate* (Lankester and Anderson 1968, Gleich et al. 1977, Upshall et al. 1986, Rowley et al. 1987, Platt 1989, Lankester and Peterson 1996, Whitlaw et al. 1996, Nankervis et al. 2000, Lankester 2001). Low infection rates imply that moose would be at a low risk of infection even with high white tailed deer densities (8-18/km^2). However, even with these low gastropod infection rates, 45% of autopsied moose carcasses collected in northeastern Minnesota showed signs of *P. tenuis* infection (Wünshmann et al. 2015).

None of the habitat variables that we measured showed a strong predictive value of gastropod density. Our habitat model included four variables but still had very low predictive power ($R^2 = 0.2245$). Because of the low variability in gastropod density and habitat measurements between cover types, we were unable to find enough variety in data to ascertain small variations in gastropod density. Some studies have been able

to find environmental variables that have an impact on gastropod densities. At larger scales climate, including humidity and temperature, was the most important factor in gastropod abundance (Maskey 2008). Grasslands and habitats with little canopy cover tend to be warmer, drier and thus less suitable for gastropods (Upshall 1986, Platt, 1987, Maskey 2008). In order to develop a stronger model, we would need to sample gastropods and make habitat measurements at a smaller landscape scale.

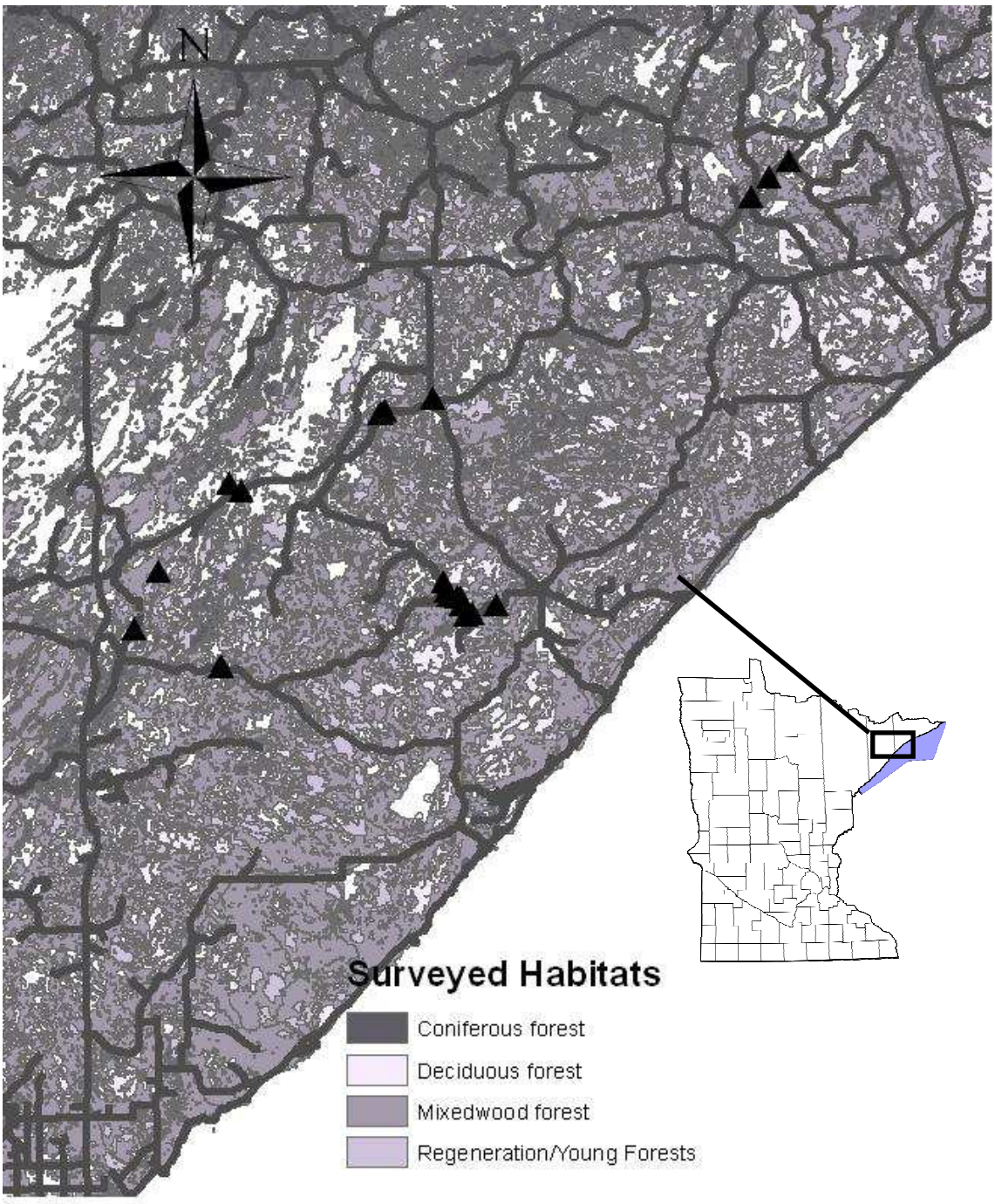

Figure 5: Sampled transect locations in northeastern Minnesota, June-November 2013 and May-August 2014. Each triangle represents a sampling transect of 10 cardboard sheets.

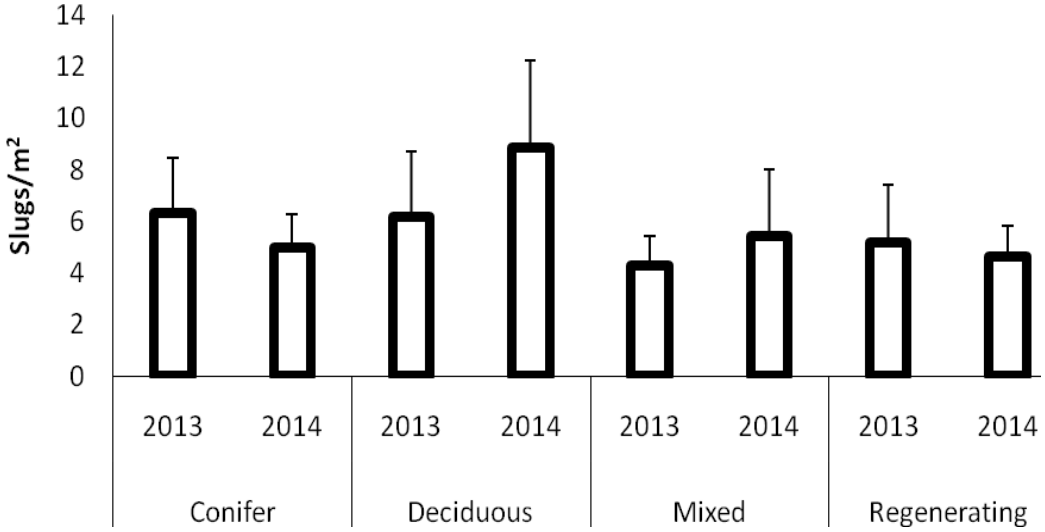

Figure 6: Slug densities in 2013 and 2014 for four habitats used by moose in northeastern Minnesota. Bars represent an average of four transects. Error bars represent Standard Error.

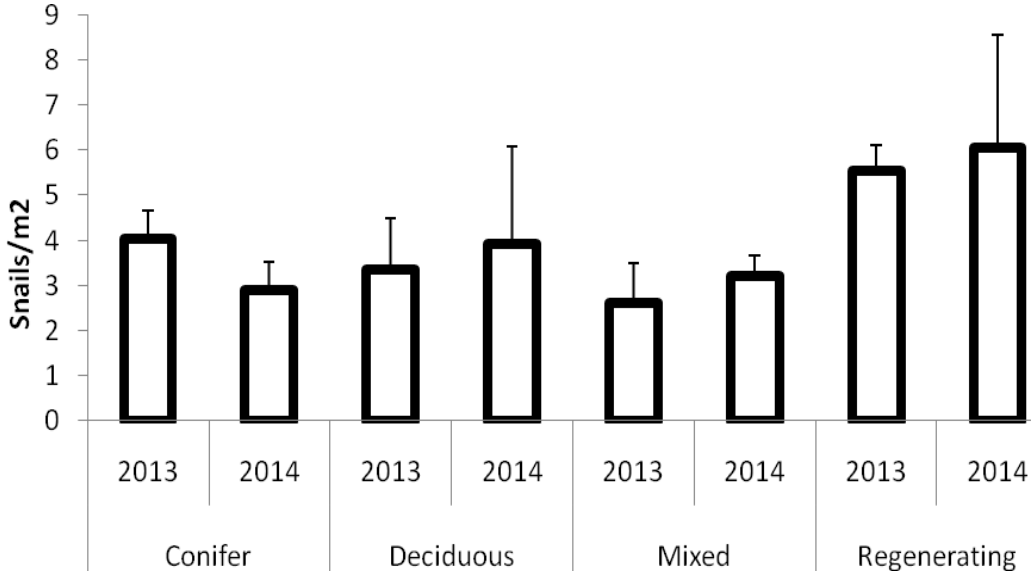

Figure 7: Snail densities in 2013 and 2014 for four habitats used by moose in northeastern Minnesota. Bars represent an average of four transects. Error bars represent Standard Error.

Table 4: Composition of terrestrial gastropod species and infected individuals in four northeastern Minnesota forest habitats, August-October 2013 and May-August 2014.

Group	Species	Infected/ Total	Coniferous Forest	Deciduous Forest	Mixed Forest	Regenerating Forest
Slug	***Arion subfuscus*** [+]	0/1,660	0/324	0/685	0/340	0/311
Slug	***Deroceras laeve*** [+]	1/463	0/107	1/107	0/121	0/128
Slug	***Arion circumscriptus***	0/188	0/44	0/122	0/15	0/7
Slug	***Deroceras reticulatum*** [+]	0/32	0/1	0/12	0/11	0/8
Snail	***Discus cronkhitei*** [+]	3/435	0/93	1/93	0/100	2/149
Snail	***Zoogenetes harpa*** [+]	0/245	0/66	0/38	0/30	0/111
Snail	***Euconulus fulvus + alderi***	0/231	0/85	0/38	0/31	0/77
Snail	***Vitrina angelicae***	0/225	0/62	0/60	0/53	0/50
Snail	***Zonitoides arboreus +nitidus*** [+]	0/200	0/74	0/33	0/16	0/77
Snail	***Cochlicopa lubrica*** [+]	0/57	0/0	0/25	0/23	0/9
Snail	***Succinea ovalis*** [+]	0/26	0/1	0/5	0/10	0/10
Snail	***Virtigo tridentate***	0/13	0/5	0/1	0/2	0/5
Snail	***Columella edentula***	0/7	0/1	0/5	0/1	0/0
Snail	***Strobilops affinis*** [+]	0/7	0/0	0/3	0/4	0/0
Snail	***Striatura ferrea***	0/3	0/1	0/2	0/0	0/0
Snail	***Anguispira alternate*** [+]	0/2	0/0	0/0	0/0	0/2
Snail	***Striatura exigua***	0/1	0/0	0/0	0/0	0/1
	Unknown Snail	649	-	-	-	-
	Unknown Slug	1,123	-	-	-	-
	Total Unknown	1,772	-	-	-	-
	Total Identified	3,795	864	1229	757	945
	% Infected	0.10	0.0	0.16	0.0	0.21

+, known *P. tenuis* host

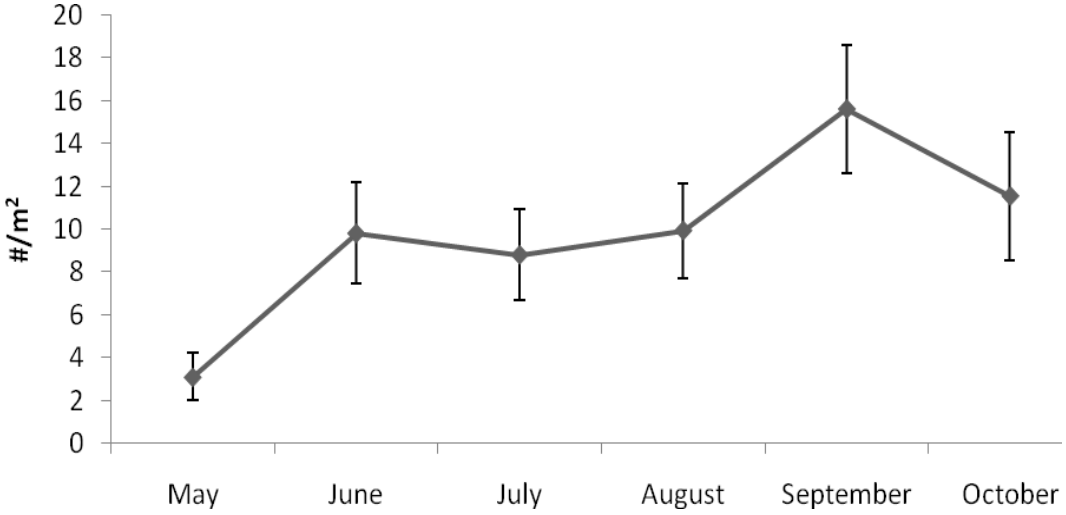

Figure 8: Gastropod densities from May to November 2013 and 2014 for all cover types. Error bars represent Standard Error.

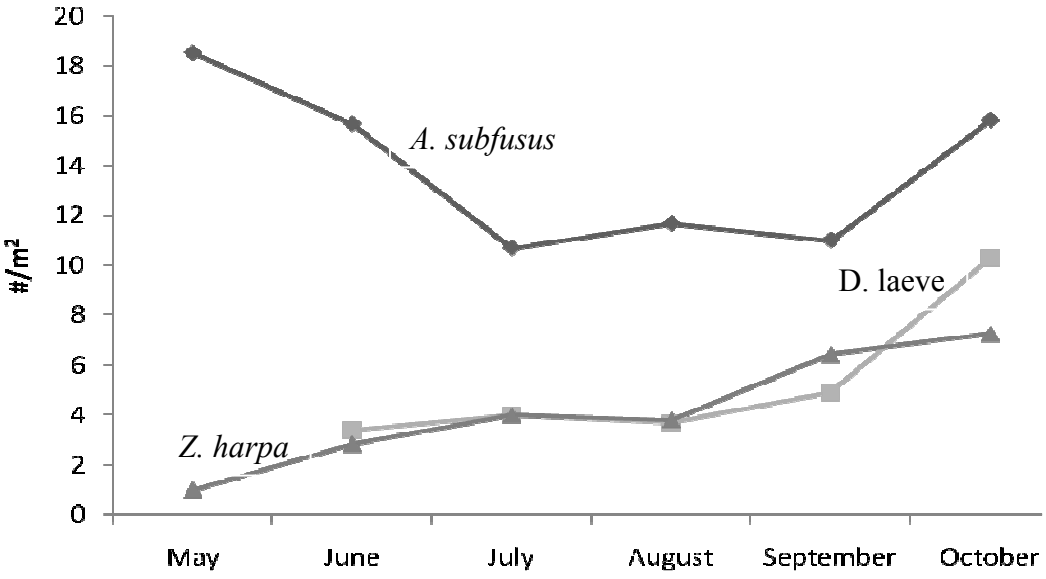

Figure 9: Changes in density of the three gastropod species that significantly changed densities from May to October 2013 and 2014: *A. subfuscus* (diamond)*, D. laeve* (square), and *Z. harpa* (triangle).

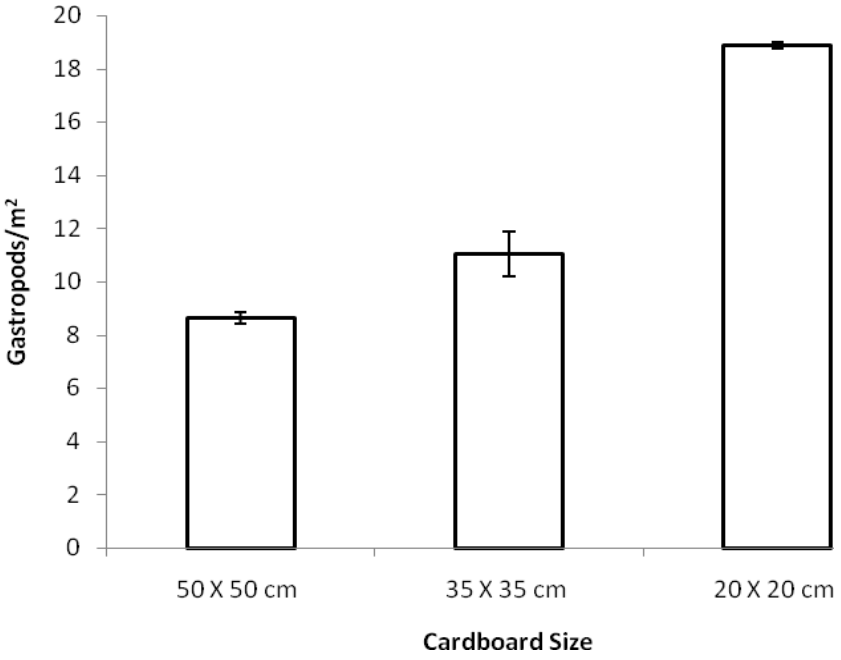

Figure 10: Estimates of gastropod density in Northeastern Minnesota forests using different cardboard sheet sizes.

Chapter 3

Parelaphostrongylus tenuis is a nematode parasite that infects white-tailed deer using gastropods as an intermediate host (Anderson and Prestwood, 1981). Three year old white-tailed deer can have infection rates up to 90% (Slomke, 1995). Infection rates in gastropods from Minnesota and Ontario are between 0.04% and 4.2% (Lankester and Anderson, 1968, Lankester and Peterson, 1996). Low infection rates in gastropods mean that primary hosts would have to eat up to a thousand gastropods in order to consume an infected gastropod (Lenarz, 2009). This would be difficult if gastropods stay in and under the litter and soil while deer browse from 0.5 to 2 m from the ground.

We performed vegetation searches to find climbing gastropods and test them for rate of *P. tenuis* infection compared to known infection rates in the area. We searched four terrestrial habitats used by radio-collared moose (deciduous forest, coniferous forest, regenerating forest, mixed forest) in Northeastern Minnesota (Lenarz, 2011). Each habitat had four transects and was sampled weekly from June to November, 2013 and May to August, 2014. All of the searches took place between 6 A.M. and 2 P.M. During sampling, the vegetation in four random 1m X 1m plots was thoroughly searched by hand to a height of 3 m. The climbing height from the litter layer, plant species and gastropod species was recorded for each gastropod found. Gastropods found on vegetation were individually digested in Petri dishes with a 1% pepsin and HCl digesting

fluid (Lankester and Peterson, 1996). We then systematically searched using a dissecting microscope (16X) for any *P. tenuis* larvae that were present in the gastropod.

A manipulation hypothesis has been proposed to explain how high infection rates in cervids can occur despite low infection rates in gastropods. Gastropod species climb both day and night in terraria but at different frequencies depending on the species (McCoy and Nudds, 1997). Infected gastropods may alter their natural behavior and climb higher into the vegetation than uninfected gastropods. In a lab study on the snail *Mesodon sayanus*, no difference in climbing height or rate was found between experimentally infected gastropods and those that were uninfected (McCoy and Nudds, 2000).

We conducted 548 field searches with 23 (4%) producing at least one gastropod. In 2013 we completed 240 field searches with 16 (7%) producing at least one gastropod. In 2014 we did 308 field searches with 7 (2%) searches producing at least 1 gastropod. We searched 548 m^2 of forest habitat and found 74 gastropods, for an average gastropod density of 0.13/m^2. 71 of the gastropods were slugs (96%) and 3 were snails (4%) (Table 5). Three species of gastropods were represented including one snail species, *Succinea ovalis*, and two slug species, *Deroceras laeve* and *Arion subfuscus* (Table 6). *A. subfuscus* was the most common gastropod (69 individuals). 54 of the *A. subfuscus* were found on June 12, 2014 in two separate deciduous forest transects located 0.5 km apart (Table 6). It is unclear why so many slugs were climbing on this day

as June is early for the usual July and August mating season of *Arion* slugs (Barnes, 1945). Dry weather provides an unsuitable environment for gastropods (Maskey, 2008), and sampling days with a capture had a higher average relative humidity (79.7%) than sampling days without a capture (70.2%) (Two-sample t-test, t = 1.90, P = 0.035). June 12, 2014 had an average relative humidity of 92%. The high average relative humidity may have provided a suitable environment for gastropods to climb higher and more frequently than usual. However, gastropods were not found climbing on all days with relative humidity >90%, and gastropods were found climbing on days with relative humidity <90%.

All but 6 of the gastropods were found on the trunks of red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), willow (*Salix* spp.), aspen (*Populus tremuloides*), or speckled alder (*Alnus rugosa*), ranging in diameter from 2 cm to 11 cm. Since moose and deer do not browse on tree trunks, the gastropods would not be ingested even if they were infected. The remaining 13 gastropods were found on the low growing herbaceous plants blue bead lily (*Clintonia borealis*) (4), raspberry (*Rubus* spp.) (1), and big leaf aster (*Aster macrophyllus*) (1). The climbing heights ranged from 3 to 300 cm (Table 5). The 3 very low climbing slugs were on the herbaceous plants and may have been crawling over the blue bead lilies rather than climbing up the plant.

No *P. tenuis* larvae (0%) were found in any of the climbing specimens. In the same transects, we found an infection rate of 0.1% in gastropods collected from the soil

and litter layer. Because of the low number of specimens collected, one infected gastropod would have resulted in an infection rate of 1.3%, almost ten times higher than the soil gastropod infection rate and the 0.08% infection rate in Northeastern Minnesota gastropods in the past (Lankester and Peterson, 1996). However, since gastropod densities can be very high in soil and litter layers, higher numbers of climbing infected gastropods would be expected if *P. tenuis* caused the gastropods to alter their behavior. Gastropod densities in the soil and litter layer of our transects were 9.9/m^2 (Cyr, 2014). We would expect 4,932 gastropods in the searched area, 1.5% of which were climbing at a given time. With 0.1% infection rates, if moose ingested every climbing gastropod (0.13/m^2), they would need to browse 7,692 m^2 of forest to ingest 1,000 gastropods. It seems unlikely for the hosts to ingest enough infected climbing gastropods to sustain *P. tenuis*. These findings suggest that *P. tenuis* does not manipulate the behavior of its intermediate host in order to facilitate transfer to final hosts, but uses a different mechanism for maintaining high deer infection rates.

Table 5: Number of slugs and snails found climbing on vegetation in four habitat types. Heights climbed were measured from the top of the litter layer.

	Slugs	Snails	Maximum Height (cm)	Minimum Height (cm)	Average Height (cm)	Median Height (cm)
Deciduous Forest	**60**	**1**	**300**	**10**	**109**	**100**
Mixed Forest	**8**	**2**	**100**	**3**	**39**	**30**
Coniferous Forest	**2**	**0**	**100**	**100**	**100**	**100**
Regenerating Forest	**1**	**0**	**20**	**20**	**20**	**20**
All Habitats	**71**	**3**	**300**	**3**	**98**	**75**

Table 6: Gastropod species and counts found climbing.

Species	# Collected
Arion subfuscus	**69**
8/8/2013	2
9/10/2013	4
9/24/2013	4
10/1/2013	2
6/12/2014	54
6/19/2014	1
7/15/2014	1
8/7/2014	1
Deroceras laeve	**2**
6/20/2013	2
Succinea ovalis	**3**
9/10/2013	2
9/24/2013	1
Grand Total	**74**

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